27th day. These observations would indicate that the roach eliminates benzpyrene following oral administration as we reported in the mouse, chicken and duck[2]. However, the rate of excretion is much slower in the roach than it is in the warm blooded animals. Certainly, there is nothing in these experiments to suggest that benzpyrene is stored in the tissues of the roach for long periods of time.

Although there is some blue fluorescence of the wings and body of the untreated roach, benzpyrene was never extracted from the body. Furthermore, the intensity of the blue fluorescence is minimal in the controls as compared with that of the roaches fed benzpyrene.

WILLIAMS[3] observed that benzpyrene, as well as some of its metabolites, has a blue fluorescence. No attempt, however, has been made in this study to demonstrate any metabolites that may be present in either the tissues or stools. Obviously, benzpyrene is metabolized within the roach since no benzpyrene was demonstrated in the stools after the food containing benzpyrene had been discontinued. However, it was still present in the body of roaches, although subsequently it disappeared. Benzpyrene remains in the tissues of the roach much longer than it does in the tissues of the mouse and chicken. These observations in the cockroach would suggest that tissues other than the kidney and liver may metabolize benzpyrene since there is no liver or kidney in the cockroach like in mammals.

Pyrene and anthracene can be extracted from the tissues of roaches fed these hydrocarbons. Pyrene and anthracene, like benzpyrene, will persist in the tissue of the roach after the food containing these 2 hydrocarbons has been discontinued. Neither of these hydrocarbons was demonstrated in the tissues of the roaches killed 17 days after food containing pyrene and anthracene had been discontinued[6].

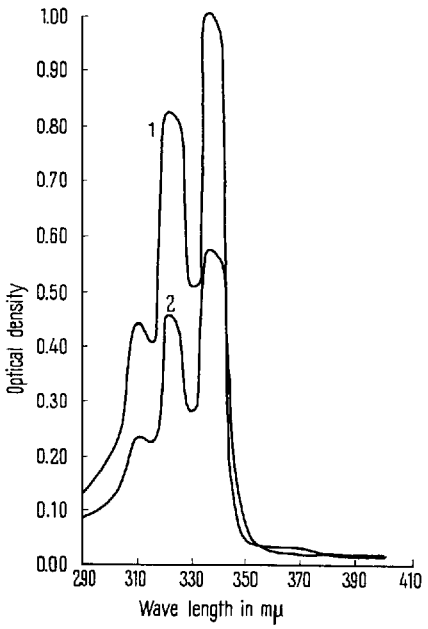

Fig. 4. Absorption curve for pyrene in tissues of roaches.

Curve 1. Pyrene standard in benzene. Tissue extracted in benzene Beckman DU Spectrophotometer

2. Body of roach fed pyrene 6 days (5 mg/g food) then control food for 7 days.

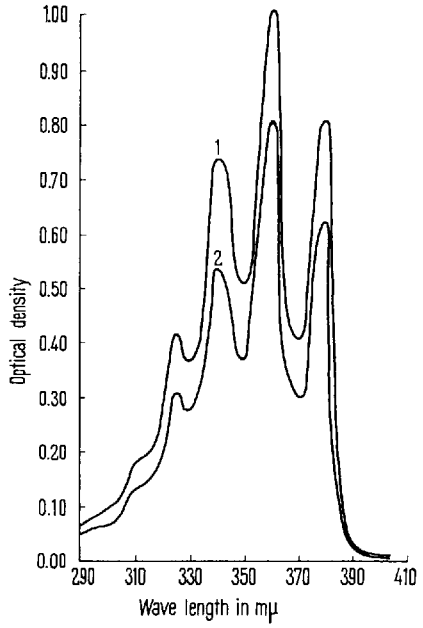

Fig. 5. Absorption curve for anthracene in tissues of roaches.

Curve 1. Anthracene standard Read in benzene. Tissue benzene extracted Beckman Spectrophotometer

2. Body roach fed anthracene 6 days (5 mg/g food) then control food for 7 days.

Résumé. Le benzopyrène le pyrène et l'anthracène peuvent être révélés au moyen du spectrophotomètre dans les extraits de tissu des blattes (*Periplaneta americana*) nourries avec ces hydrocarbures; ils sont excrétés lorsqu'on interrompt l'alimentation.

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Departments of Pathology and Preventive Medicine and Public Health, The University of Texas Medical Branch, Galveston (Texas, U.S.A.), May 27, 1963.

[6] This study in the cockroach was suggested by J. MACK, a laboratory assistant, who also contributed much to the technical conduct of the experiment.

Effect of Ionizing Radiation on the Testes of Rat

X-irradiation has a marked effect on the living germ cells, as evidenced by the various changes in the constitution and behaviour of the intracellular inclusions. The sequence of these aberrations is difficult to follow as chronological studies of the living irradiated germ cells have not been carried out in the present investigations and will be dealt with in detail elsewhere.

Pure bred Wistar rats, eight weeks old, were subjected to whole body irradiation according to the following schedule – 325 r at 200 kV, filter 1 Al plus $^1/_2$ Cu, target distance 50 cm, with the dose rate at 36 r/min. The initial dose of 325 r was repeated every 24 h till the maximum dose reached 1300 r. Control rats from the same age group were kept and provided with the same experimental conditions without exposure to X-rays. Induced rats from different dose levels were sacrificed after a week and

smears of testes were prepared and fixed in formol calcium[1]. The staining was carried out by Hollande's Chlorcarmine, iron alum haematoxylin, and eosin-haematoxylin methods[2].

The changes in the irradiated rats were slow and gradual and were hardly perceptible at lower dose levels. At 1300 r both the external and internal changes in the testes were pronounced. The present investigations deal with the changes at the 1300 r dose level but the conclusions drawn will apply equally well to any dose which causes external morphological changes and the involution of testis.

Among marked external changes in the body were the gradual opacity of the lens of the eye, general swelling of the face, animals becoming sluggish and ultimately dying after a short lapse of time. Just prior to the death, the testes were dissected out and smears were prepared.

Cytological examination of the normal smears revealed the presence of Sertoli cells with prominent nucleus marking the peripheral lining and the oblong interstitial cells. Both Sertoli and interstitial cells were active and mitotic divisions were observed. The lumen of the testis was full of spermatogonia and the secondary spermatocyte. There was no trace of any of the advanced stages of spermatogenesis. Both the spermatogonia and the spermatocytes were in growth phases of the meiotic divisions indicating the initiation of the spermatogenesis. The spermatogonia contained rounded or oval Golgi bodies which became more pronounced in the secondary spermatocytes having a chromophobe interior and chromophilic exterior. They were mainly distributed along the lateral aspects of the nucleus enclosing a dark stained granule, the centrosome which was present towards the anterior end of the nucleus. The mitochondria were in the form of small rodlets and scattered throughout the cytoplasm. The interphase nucleus was bound by a thin transparent membrane enclosing the chromatin network with a prominent nucleolus.

In irradiated specimens, various abnormalities had been observed. The effect was significantly little on the outer cellular layer which consisted mainly of Sertoli cells. The noticeable change was the cessation of mitosis and the clumping of nuclear material. The interstitial cells were comparatively radioresistant and showed no, or very little, variation. In some interstitial cells where mitosis was initiated, it was completed without any aberration in spite of heavy irradiation.

The Golgi bodies, which were circular in outline in the normal spermatocytes, assumed granular appearance in the irradiated specimens (Figure 1) and became concentrated as granular mass. The centrosome was also divided into two, moved towards the posterior aspect of the nucleus and occupied the position near the mitochondrial mass (Figure 1). Further changes in the centrosomes could not be followed due to their extremely small size.

The mitochondrial rodlets showed peculiar aberration in irradiated rats. They began to aggregate and agglutinate, and occupied a juxta nuclear position (Figure 2). The individual identities of the rodlets were lost and the entire mitochondrial element appeared as a single mass resembling that of irradiated mitochondrial 'nebenkern' of insects[3]. This clearly indicated a lag effect as, in the normal spermatogenesis, this condition is met with in the spermatid stage.

The first changes in the spermatocyte which appeared due to irradiation were found in the nucleus. A short time after irradiation, the clumping of the chromatin material took place. Other alterations included the vacuolization of the chromatin material and the nucleolus, the stoppage of all division stages, and the thickening of the nuclear membrane. None of the structures of the nucleus, however, lost the staining properties (Figure 2).

There is a general agreement that all cell inclusions are sensitive to X-rays[4,5], and show abnormal changes. There are two views regarding the sensitivity of the cell inclusions. According to Fogg and Warren[6], Bloom and Bloom[7], and Spear[5], the nucleus is more sensitive than the rest of the cell; while the opposing view is held by Duryee[8] and Carlson[4], suggesting cytoplasmic inclusions to be more susceptible to X-rays. The present investigations support the former view since the nuclei of various cells tend to be affected even after small doses of X-rays, and the effect is indicated by the rapid cessation of mitosis and the clumping of the chromatin material. Warren[9] suggested that not all mitosis is inhibited due to X-rays. Most cells which had undergone the first morphologically evident mitotic changes seem to go through the rest of the process although they may show lagging or otherwise damaged chromosome. Such an observation was true only for the interstitial cells in our investigations in some of which the mitosis continued.

The spermatocytes show a general lagging effect, the accumulation of the mitochondria and the conversion of the Golgi bodies into the granular form are due to irradiation effects. This effect on the Golgi bodies had been observed earlier by Gatenby and Wigoder[10] in X-irradiated *Cavia* specimens, but they were unable to state definitely that this was an irradiation effect. The movement of the centrosome from the Golgi complex to the mitochondrial region and its division is due to the fact

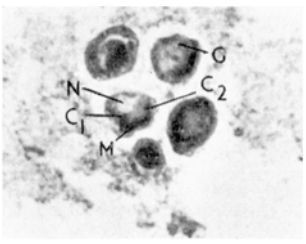

Fig. 1

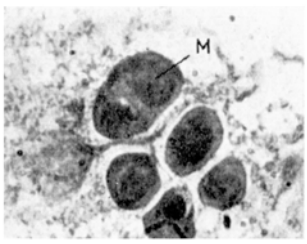

Fig. 2

Fig. 1. Smear of irradiated testis showing secondary spermatocytes with abnormal nucleus (N), aggregated mitochondria (M), dividing centrosomes (C_1 and C_2), and Golgi granules (G). × 670.

Fig. 2. Smear of irradiated testis showing spermatocytes with agglutination of mitochondria (M). × 670.

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that the Golgi bodies lose their form, leaving the centrosome free. The movement and subsequent division is enhanced due to irradiation. It has also been suggested by LINDERGREN et al.[11] that the centriole is a primary radiation target. Since the centrosome encloses the centrioles, and if the latter are radiation target, the movement and division of centrosome could easily be explained. As stated earlier, due to the small size of this body, no positive explanation could be offered[12].

Zusammenfassung. Röntgenstrahlen haben einen bemerkenswerten Einfluss auf die Inklusionen der männlichen Geschlechtszellen, wie er durch die Verklumpung des Chromatins, die Aggregation der Mitochondrien und die Granulanatur des Golgikörpers evident wird.

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Department of Zoology, University of Rajasthan, Jaipur (India), April 22, 1963.

[11] C. C. LINDERGREN, D. D. PITTMAN, and A. YUASA, Trans. N.Y. Acad. Sci. *21*, 524 (1959).

[12] We wish to thank Prof. L. S. RAMASWAMI for various facilities in the Department. Our thanks are also due to Dr. D. G. OJHA and Dr. K. N. PANJAL for the irradiation facilities in the M.G. Hospital, Jodhpur.

Changes in Protein-bound Sulfhydryl Group Concentration in the Liver of Rats Fed Cabbage

The effect of goitrogenic substances on tissues in which the oxygen utilization becomes reduced is well known, and it may be expected that the goitrogenic agents will affect the level of sulfhydryl compounds in various organs of experimental animals. We have found a marked increase in the total sulfhydryl compounds in the liver of rats after feeding with goitrogenous cabbage[1]. These results were an incitement to a further study of the problem.

Material and Methods. In the present experiment 20 white female rats of the Wistar strain were fed winter cabbage (*Brassica oleracea* var. capitata) for 180 days. In addition, they received Larsen's diet, but no water. (Average daily consumption per rat was about 35 g of cabbage and 7 g of Larsen.) The controls, a group of 10 rats, had Larsen's diet and water *ad libitum*. At the end of the experiment, the rats were sacrificed under ether anaesthesia by a puncture of the ventral aorta, and the relative weight of the thyroid and of the liver was determined. The liver was weighed immediately on being taken out and total sulfhydryl compounds and non-protein sulfhydryl compounds were determined by polarographic estimation. The difference between total sulfhydryl compounds and non-protein sulfhydryl compounds represented the value of protein-bound sulfhydryl groups. 2% sulfo-salicylic acid were used for the deproteinization of liver homogenates. In addition, SH-glutathione was determined in the deproteinized liver homogenates and in hemolysed and deproteinized blood by the manometric method with the aid of glyoxalase, prepared from baker's yeast. The enzymatic estimation of SH-glutathione was done according to WOODWARD's method[2]. All sulfhydryl

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Determinations performed in the experiment

Type of estimation	Experimental group E (n = 20)[a]	Controls C (n = 10)	Significance of difference
Thyroid weight in mg/100 g body weight	9.01 ± 1.77[b]	6.76 ± 0.49	E > C significant $p \lessapprox 0.001$
Liver weight in g/100 g body weight	3.71 ± 0.31	3.44 ± 0.34	E > C non-significant
Thiocyanate in serum according to Aldridge, in mgm% SCN^{-1}	1.08 ± 0.19	0.36 ± 0.10	E > C significant $p \lessapprox 0.001$
Total sulfhydryl compounds in liver estimated polarographically	520.60 ± 155.50[c]	372.30 ± 68.30	E > C significant $p \lessapprox 0.02$
Protein bound sulfhydryl groups in liver	337.6 ± 123.90	193.80 ± 68.70	E > C significant $p \lessapprox 0.01$
Non-protein sulfhydryl compounds in liver estimated polarographically	187.70 ± 27.50	176.20 ± 19.10	E > C non-significant
SH-glutathione in liver estimated enzymatically	188.20 ± 43.30	195.40 ± 38.60	C > E non-significant
SH-glutathione in blood estimated enzymatically	40.96 ± 7.34	43.40 ± 8.46	C > E non-significant

[a] n = Number of rats in the experiment. [b] $(\bar{X} + \sigma)$ = Average + standard deviation. [c] All sulfhydryl compound fractions are expressed in mg% of SH-glutathione in fresh liver or blood.